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## Survival of *Salmonella* and *Escherichia coli* in pig slurry: simulation of decay

**PMP 01**

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**Summary:** Spreading of slurry infected with multi-resistant *Salmonella* Typhimurium DT104 (MRDT104) on arable land might constitute a risk of transmission to wildlife. To estimate survival time on farmland, we modeled the bacterial decay based on *Escherichia coli* data from a plot study carried out in spring 2002 in Denmark. Time until undetectable levels were modeled under different scenarios: 1) *E. coli* in swine slurry, 2) *Salmonella* in slurry from clinically infected swineherds, and 3) MRDT104 in slurry from sub-clinically infected swineherds. A log-linear model extended with time$^2$ and time$^3$ was used to describe bacterial decay. For scenarios 2 and 3, we assumed that the level of bacteria in the slurry would be log 4.0 cfu/g and log 3.4 cfu/g, respectively, and a similar effect of spreading and decimation to that of *E. coli*. Hereby, it was estimated that *Salmonella* counts fell below detectable levels after 10 and 5 days, respectively.

**Keywords:** microbial ecology, transmission, multi-resistant *Salmonella* Typhimurium DT104, environmental persistence, decimation

**Introduction:** Spreading of slurry infected with multi-resistant *Salmonella* Typhimurium DT104 (MRDT104) on arable land has been considered a potential hazard for transmission to wildlife. Therefore, spreading has been restricted for herds positive to MRDT104. Our aim was to model decay of *Escherichia coli* and *Salmonella* after spreading of contaminated slurry on farmland, and to estimate the survival time.

**Materials and Methods:** The modeling was based on *Escherichia coli* data from a plot study carried out in spring 2002 in Denmark. Here, *E. coli* was measured quantitatively on day 0, 7, 14, 21, and 28 after application on soil using 4 different methods (see Boes & Alban, in this issue). *Salmonella* was not detected when slurry was ploughed in, and hence, these data were not used for the modeling. Data from the three remaining application methods (harrowed only, slurry injection, and hose application) were used.
We were interested in estimating the time from disposal until undetectable levels under different scenarios: 1) *E. coli* in swine slurry, 2) *Salmonella* in slurry from clinically infected swineherds, and 3) MRDT104 in slurry from sub-clinically infected swineherds.

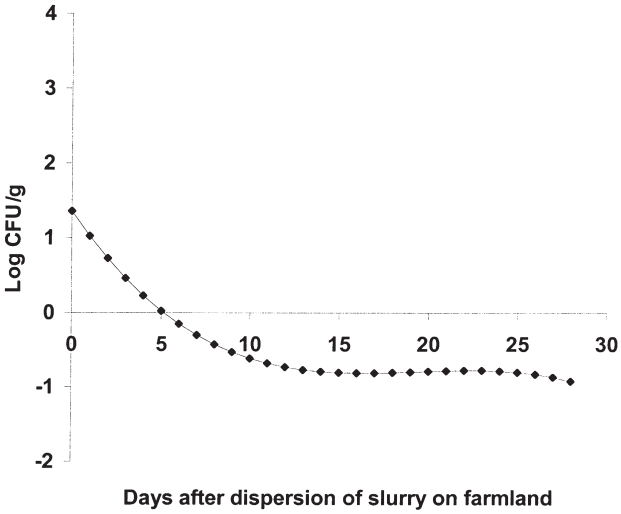


**Fig. 1:** *Modeled decay of multi-resistant Salmonella Typhimurium DT104 in slurry from sub-clinically infected swineherds based on E. coli data from a Danish plot study, carried out in 2002. Detection level for Salmonella was 1 cfu/g = 0 Log.*

A log-linear model extended with time$^2$ and time$^3$ was used to describe bacterial decay. The detection level was log 1 cfu/g for *E. coli* and log 0 cfu/g for *Salmonella* (enrichment phase included in laboratory procedure).
For scenarios 2 and 3, we assumed that the level of bacteria in the slurry tank would be log 4.0 cfu/g and log 3.4 cfu/g, respectively. Furthermore, we assumed a similar effect of spreading and decimation to that of *E. coli*.

**Results:** The following model was found to be the best at explaining the decay of *E. coli*: log(*E. coli*)= 3.2150-0.3508*time+0.0186*time$^2$-0.0003*time$^3$ ($r^2$=81%). According to this model, *E. coli* levels reached the detection limit after 15 days.
For *Salmonella* (scenario 2 and 3) it was estimated that the bacterial counts fell below detectable levels after 10 and 5 days (Fig. 1), respectively.

**Discussion:** *E. coli* was used instead of *Salmonella*, because it occurs in much higher numbers, and its decimation time ($T_{90}$) is comparable. $T_{90}$ for *E. coli* and *Salmonella* in our model were similar to $T_{90}$ obtained from observational studies. The level of *E. coli* was representative for swine slurry, as were the assumed levels of *Salmonella* in slurry from clinically and sub-clinically infected swineherds.
Our findings are in agreement with results from observational studies, e.g. the plot study, where *Salmonella* was detected in soil samples until day 7, where 1 sample out of 32 was positive (Boes & Alban, in this issue).
The results are contrary to results from experimental studies. This might be because much higher bacteriological levels are used in experimental studies. Furthermore, in experimental set-ups it is difficult to capture the multi-factorial nature of the biological processes influencing bacterial survival. Finally, our study estimated the average decline of *Salmonella*, without aiming to show when all viable counts had truly disappeared.

**Conclusion:** We conclude that MRDT104 levels in slurry from sub-clinically infected swineherds are below detectable levels less than 10 days after application on farmland. The low detection limit for *Salmonella* implies, that hereafter, only a negligible concentration of MRDT104 is left in the soil, lowering

the risk of transmission to wildlife. Until the 10 days have passed, MRDT104 bacteria are present, and as such constitute a potential hazard. However, as it is not allowed to spread slurry from MRDT104 infected herds on pasture or ready-to-eat vegetables, there is no direct exposure to grazing stock or humans.

## Risk analysis of *Bacillus* spp. isolated from cured pork sausages

**PMP 02**

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**Summary:** This study was undertaken to acquire information about the toxigenic potential of *Bacillus* strains isolated from eight cured pork sausages obtained from traditional or industrial processings. The application of RAPD-PCR protocols made it possible to identify 52 different biotypes among 220 heat-resistant Gram-positive endospore-forming colonies. The sequence analysis of the 5′ region of 16S rDNA revealed that 36 strains belonged to *B. subtilis* and 16 to *B. pumilus* species. No strains belonging to *B. cereus* species were isolated from the cured sausages analysed. The toxigenic potential of these strains was assayed by PCR analysis and physiological tests to identify the most important *B. cereus* toxins and virulence factors. No specific PCR fragment was obtained from any of the strains; however, some of them were found positive for hemolytic and lecithinase activity. These preliminary results reassure about the microbiological risk related to the presence of pathogenic *Bacillus* strains in cured pork sausages analysed even though the hemolytic and lecithinase activities found in some strains suggest that more in-depth analyses need to be carried out.

**Keywords**: PCR, toxins, virulence factors, *B. cereus*, cured meat products

**Introduction:** A wide variety of microorganisms such as, lactic acid bacteria (LAB), *Staphylococcus*, *Kocuria* and *Bacillus* are involved in meat fermentation. Most cases of food poisoning attributed to *Bacillus* species are associated with *B. cereus*; this bacterium is known to cause a variety of foodborne disorders characterized by either diarrhea or emesis. Lately, other *Bacillus* species have been gaining recognition as organisms relevant in causing food poisoning, with recent epidemiological evidence linking *B. subtilis, B. pumilus*, *B. licheniformis*, and *B. thuringensis* with incidents of foodborne illness. Evaluation of toxin gene presence and expression in *Bacillus* spp. other than *B. cereus* has not been thoroughly investigated. The survival of *Bacillus* strains through meat processing leads to suppose that potentially pathogenic ones could be present in cured sausages.
For these reasons we analyzed *Bacillus* strains isolated from industrial and traditional cured pork sausages to gain insight into their potential role in foodborne infections.

**Material and Methods:** Eight sausage samples were collected from the local market; the sausage casing was removed aseptically and 20 g sample from the central portion of each sausage was homogenized (10 % w/w) in a saline solution. Five milliliters of cell suspension were pasteurized at 80 °C for 10 min and then cooled to room temperature. Serial decimal dilutions in 0.1 % peptone water were poured onto non-selective tryptose soy agar plates (Oxoid, Basingstoke, UK). Aerobic mesophilic counts were determined after incubation at 30 °C for 72 h. Thirty colonies from each sample were collected and analyzed for Gram stain, cell morphology, presence of endospores and catalase reaction. The genomic DNA of each isolate was extracted with DNA Purification Kit (Promega, UK). The isolates were biotyped and taxonomically identified by using a two-step RAPD-PCR protocol and 16S rDNA sequencing (Baruzzi et al., 2000). By means of PCR assays, the strains were analysed for most